EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1251

NIST-TRACEABLE NMR METHOD TO DETERMINE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NITROGEN MUSTARD HN-1 FEEDSTOCK SAMPLES

David J. McGarvey

RESEARCH AND TECHNOLOGY DIRECTORATE

William R. Creasy

LEIDOS
Gunpowder, MD 21010-0068

June 2014

U.S. ARMY RDECOM

Disclaimer

The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED *(From - To)***
XX-06-2014	Final	Jan 2012 - May 2014

4. TITLE AND SUBTITLE

NIST-Traceable NMR Method to Determine Quantitative Weight Percentage Purity of Nitrogen Mustard HN-1 Feedstock Samples

5a. CONTRACT NUMBER
W911SR-10-D-0004

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S

McGarvey, David J.; and Creasy, William R. (Leidos)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Director, ECBC, ATTN: RDCB-DRC-M, APG, MD 21010-5424
Leidos, P.O. Box 68, Gunpowder, MD 21010-0068

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1251

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Director, ECBC, ATTN: RDCB-DPC-RQ, APG, MD 21010-5424

10. SPONSOR/MONITOR'S ACRONYM(S)
ECBC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release, distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A NIST-Traceable method for using NMR with proton detection is described to determine the weight percent purity of feedstock samples of nitrogen mustard, HN-1.

15. SUBJECT TERMS

Nitrogen Mustard HN-1 Purity Determination Nitrogen Mustard NMR

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					Renu B. Rastogi
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER *(include area code)***
U	U	U	UU	28	(410) 436-7545

Blank

PREFACE

The work described in this report was authorized under Contract No. W911SR-10-D-0004. This work was started in January 2012 and completed in May 2014.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of endorsement.

The text of this report is published as received and was not edited by the Technical Releases Office, U.S. Army Edgewood Chemical Biological Center.

This report has been approved for public release.

Acknowledgments

The authors thank Sue Procell (Directorate of Program Integration, U.S. Army Edgewood Chemical Biological Center) for funding this project.

Blank

CONTENTS

1. INTRODUCTION 1

2. PROCEDURE 1

2.1 Supplies 1
2.2 Sample Preparation 2
2.3 Obtaining an NMR spectrum 3
2.4 Data Processing 4
2.5 Purity Calculation 4

3. CONCLUSIONS 5

LITERATURE CITED 8

APPENDIX: PRECISION AND ACCURACY TESTING DATA FOR THE HN-1 METHOD 9

FIGURES

1. Proton NMR spectrum of HN-1 agent and the internal standard 1,2,4,5-tetramethylbenzene 6

2. Plot showing the spectrum in Figure 1 with an expanded y-scale 7

I-1 Plot of inversion recovery data for IS 10

I-2 Plot of the data from 4 days, Found Z vs. Target Z 13

TABLES

I-1. Data from Day 1.....11

I-2 Data from Day 2.....11

I-3 Data from Day 3.....12

I-4 Data from Day 4.....12

I-5 Data from Day 1, used to calculate purity for each run.....14

I-6 Data from repeated runs of one prepared sample 15

Blank

NIST-TRACEABLE NMR METHOD TO DETERMINE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NITROGEN MUSTARD HN-1 FEEDSTOCK SAMPLES

INTRODUCTION

This procedure is based on previously published Technical Report procedures for using Nuclear Magnetic Resonance (NMR) instruments for determining the purity of CW agent samples.[1,2,3,4,5,6] The procedure utilizes an internal standard to establish an absolute weight percentage for the analyte of interest. Identifying the structures or other components in the mixture is not necessary. All that is necessary is to know the NMR chemical shifts of the major analyte and the internal standard, and the molecular weights. The weight percent calculations are not negatively affected by the presence of undetectable components in the sample (e.g. inorganic salts, insoluble solids, etc.).

The compound nitrogen mustard HN-1 is ethyl-bis(2-chloroethyl)amine. The procedure, using a NIST standard material, is very similar to a previous method for HN-3.[6] Modifications have been made to the procedure to account for differences in the data acquisition and processing. As with the previous method, an internal standard was purchased from Sigma Aldrich that has NIST-traceable purity, and a balance that is calibrated with NIST-traceable weights is used. These modifications make the method into a NIST-Traceable method.

In order to determine the purity of nitrogen mustard-1 (HN-1), proton NMR is used for detection.

1. PROCEDURE

2.1 Supplies

The following supplies can be used for the procedure. Equivalent supplies may be available from other vendors.

The internal standard was 1,2,4,5-tetramethylbenzene, purchased from Fluka (Sigma Aldrich), Part Number 74658-5G, CAS No. 95-93-2, as a TraceCERT® certified reference material (CRM) standard for quantitative NMR.

The following supplies were purchased from Wilmad, 1172 NW Boulevard Vineland, NJ 08360, phone 800-220-5171 (http://www.wilmad-labglass.com/ordering/index.jsp):

Item	Part Number
5 mm dia. 8" long NMR tube	WG-1000-8-50
Teflon inserts	6005
Pasteur pipets, 9"	C-7095B-9

The following supplies were purchased from Sigma Aldrich (http://www.sigmaaldrich.com/chemistry.html):

Item	Part Number
Chloroform, 99.9% D	23,689-6

For the Precision and Accuracy testing, a JEOL ECS-400 Nuclear Magnetic Resonance spectrometer with a 400 MHz (9.8 T) magnet and 5 mm liquid analysis probe was used. A Sartorius Cubis balance (Model MSA6.6S-000-DM, precision 1 µg) was used after installation in a fume hood and calibration using NIST-traceable weights. NMR systems and balances from other vendors should give comparable results, if the operators have the appropriate training and appropriate QC procedures are used.

Other common laboratory equipment will be used, including a vortex mixer, spatulas, and volumetric pipets. This equipment is not critical to the accurate performance of the method.

2.2 Sample Preparation

This procedure is performed under appropriate engineering controls, in accordance with appropriate surety and safety regulations, equipment validations, and SOPs approved by the Safety and Health Office. The balance must be calibrated using NIST-traceable weights.

a. Tare a screw-cap vial with cap on the balance. Transfer a quantity of 10-20 mg of neat internal standard, NIST traceable 1,2,4,5-tetramethylbenzene, into the vial. This compound is a solid material, so it is transferred with a spatula. Replace the cap and determine the weight of the internal standard to an accuracy of 0.01 mg. Tare the balance after recording the weight.
b. Add 5-35 mg of feedstock agent sample to the vial. The liquid agent can be measured with a pipet (4.6 to 32 µl of liquid, using a density of 1.09 g/ml). (A precision and accuracy test of this method has been done over this range of agent amounts, see Appendix I.) Record the weight to an accuracy of 0.01 mg in a laboratory notebook. Appropriate agent accountability documentation is used to record the consumption.
c. Add 0.5 ml of reagent grade deuterated chloroform ($CDCl_3$).

d. Vortex or mix the sample for at least 15 s to dissolve both compounds in the solvent.
e. Transfer the solution into a PTFE NMR tube insert. (OPTIONAL: A glass 4mm insert tube or capillary tube may be used, and flame sealed, if desired)
f. Place the insert into a 5 mm glass NMR tube and push it to the bottom of the tube. Cap the insert with a PTFE stopper. Cap the NMR tube with a cap.

2.3 Obtaining an NMR spectrum

Operators of the NMR must have sufficient training from the instrument company representatives to understand the general operational principles and to use the instrument computer control to perform the required tasks. The instrument documentation can be consulted for detailed instructions. In order to validate the NMR is functioning correctly, it is suggested that a manufacturer sample such as 0.01% ethylbenzene in deuterated acetone can be analyzed to check the signal response on a continuing basis. This analysis can be done periodically as part of the instrument QC validation, but detailed QC specifications are not included in this method.

a. Place the NMR tube into the spinner using a depth gauge to orient the tube at the correct position. Lower the sample into the magnet bore. (Note: The doubly-contained NMR tube that contains agent will be outside of engineering controls, and this situation may need to be addressed in the SOP.)
b. Lock the instrument on the deuterium signal from the $CDCl_3$.
c. Shim the magnet to maximize the lock signal.
d. Tune and match the probe. (On some instruments, this operation is done automatically by the instrument software and autotune equipment. On older instruments, it must be done using manual adjustments on the NMR probe.)
e. OPTIONAL: Determine the T_1 relaxation time of the analytes in the sample solution. Use the instrument console to load the data file or instrument parameters for an inversion recovery experiment for proton detection. Perform the experiment with at least 6 delay times. Process the data to plot the recovery curve for each analyte peak, and determine the T_1 relaxation time from the data plot. Identify the longest T_1 value for all the peaks. The relaxation delay time for the quantitative purity measurement is calculated to be at least 10 X the longest relaxation time. This procedure to determine the T_1 relaxation time should be done if there is an inconsistency in the purity determination, if a new instrument is being used, or if it is necessary to minimize the experiment acquisition time.
f. Load instrument parameters to acquire a 1D proton spectrum. If the T_1 relaxation time is not determined (step e is not performed), then set the relaxation time to 40 s. (This is typically 10 X longer than the longest T_1 in the solvent.) Do not use NOE, decoupling, or water peak suppression.

g. Open a new data file on the NMR computer with a unique filename and the sample information and notebook reference, and using parameters for proton acquisition. If a parameter set for purity determinations has already been saved, it can be opened. The following parameters are used. (Actual parameter names will vary depending on the make and model of the NMR and can be found in the NMR documentation.):

    Relaxation time: 40 s or as determined in step e.
    Excite pulse: 90º pulse (determining the time for this pulse should be found in the NMR instrument documentation)
    Number of data points: 64K
    Sweep width: 15 ppm
    Center frequency: 5 ppm
    Decoupling: off
    NOE: off
    Automatic gain determination: on

h. Acquire data.
i. A total of 7 or more replicate runs are acquired for statistical determination of the NMR variability, signal to noise ratio, and integration errors. Several samples can be prepared to determine the weighing statistical errors (see Appendix I).

2.4 Data Processing

Data must be processed after the acquisition by individuals training in the use of the instrument software.

a. Apply a window function (exponential multiplication). This may be done using a line broadening parameter of 0.5 to 2 Hz. The parameter can be adjusted to enhance the signal to noise ratio. A larger line broadening produces wider peaks, which can degrade the resolution between peaks. The same value of line broadening must be used for all the data files.
b. Fourier transform (FFT) to convert data from time to frequency domain and produce the NMR spectrum. A sample spectrum is shown in Figure 1.
c. Phase all peaks in the spectrum and correct the baseline if necessary.
d. If necessary for reporting, reference the chemical shift against the internal standard.
e. Integrate the relevant peaks in the spectrum to obtain the areas. A sample integrated spectrum is shown in Figure 2 with an expanded y-scale. Some data systems will perform automatic integration of peaks. It is necessary for the operator to examine the integration to make sure that the appropriate parts of the peak are included in the integration. If the integration is incorrect, the spectrum can be manually integrated. In particular, Figure 2 shows that each peak has two ^{13}C satellite peaks on each side of the main peak. These peaks are produced by molecules that have a natural abundance of ^{13}C

isotopes, and they each represent 0.55% of the center peak. The satellite peaks should be included in the integration of the central peak. (If the magnet is not well shimmed, the satellite peaks may not be resolved.)

2.5 Purity Calculation

The weight percent of the analyte (Wt% A) in the sample is calculated using the following formula, where analyte A is the HN-1, and IS is the internal standard:

$$\text{Wt\% A} = \frac{\text{Area under A peak}}{\text{Area under IS peak}} \times \frac{\text{MW of A}}{\text{MW of IS}} \times \frac{\text{Weight IS}}{\text{Weight A}} \times \frac{\text{No. identical H(IS)}}{\text{No. H (A)}} \times 100\%$$

Area under A peak = the area of the triplet peaks at 3.5 ppm and the ^{13}C satellite peaks that are associated with them;
Area under IS peak = total area of the singlet peak at 2.2 ppm and the ^{13}C satellite peaks;
MW of A = average molecular weight of HN-1, which is 170.08 D;
MW of IS= average molecular weight of the internal standard, which is 134.22 D;
Weight IS=balance recorded weight of internal standard in the vial;
Weight A=balance recorded weight of feedstock HN-1 sample in the vial;
No. identical H (IS)=the number of identical protons in the internal standard, which is 12;
No. H (A)=the number of protons in the integrated peaks of the analyte, which is 4 for the triplet peaks at 3.5 ppm.

The purity of the internal standard can be found on the documentation from the NIST traceable IS.

If the analytical statistical accuracy is reported, the calculated weight percentages for each replicate run can be averaged to find a mean (average) and standard deviation. For 7 replicates, the mean ± 2 × standard deviation provides the 95% confidence range.

2. CONCLUSION

By using the NIST-traceable internal standard, and the balance that is calibrated with NIST-traceable weights, the purity of the CW agent feedstock HN-1 is determined using a NIST-Traceable method.

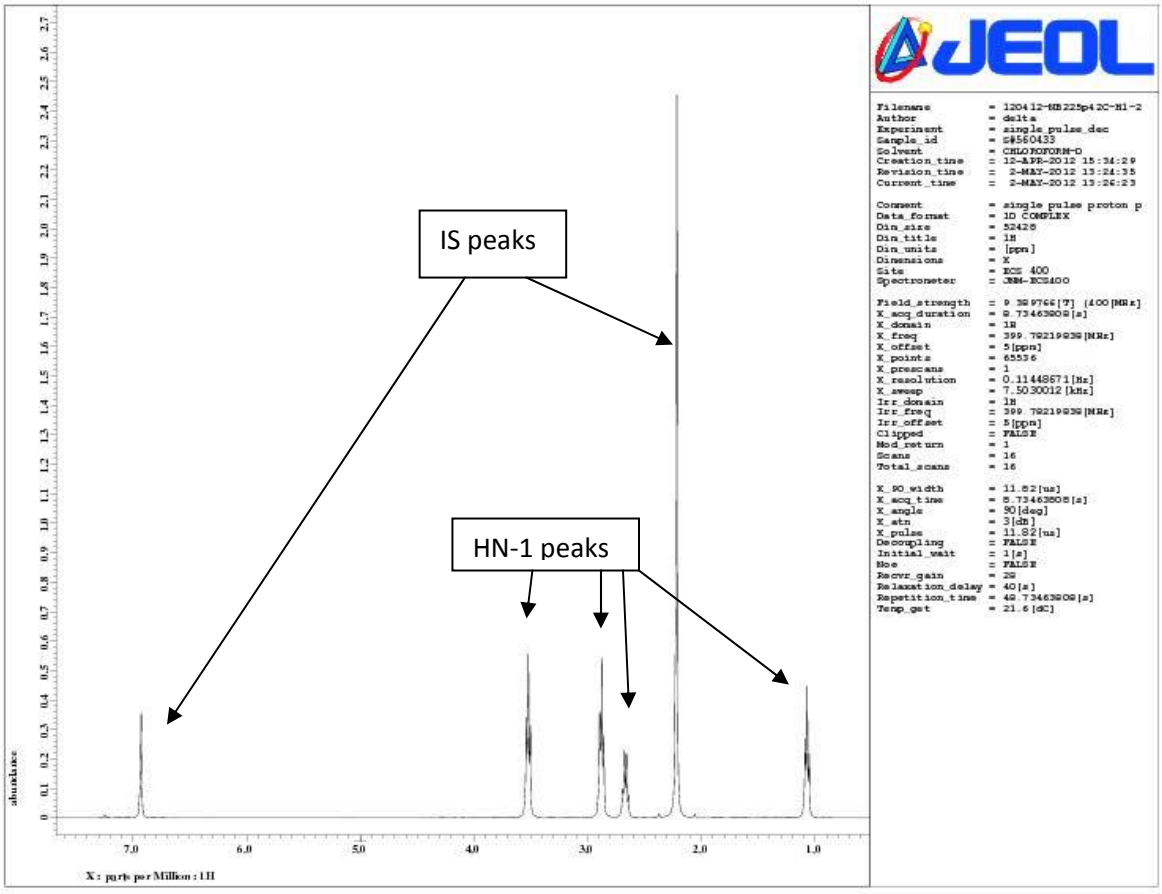

FIGURE 1: Proton NMR spectrum of HN-1 agent and the internal standard 1,2,4,5-tetramethylbenzene.

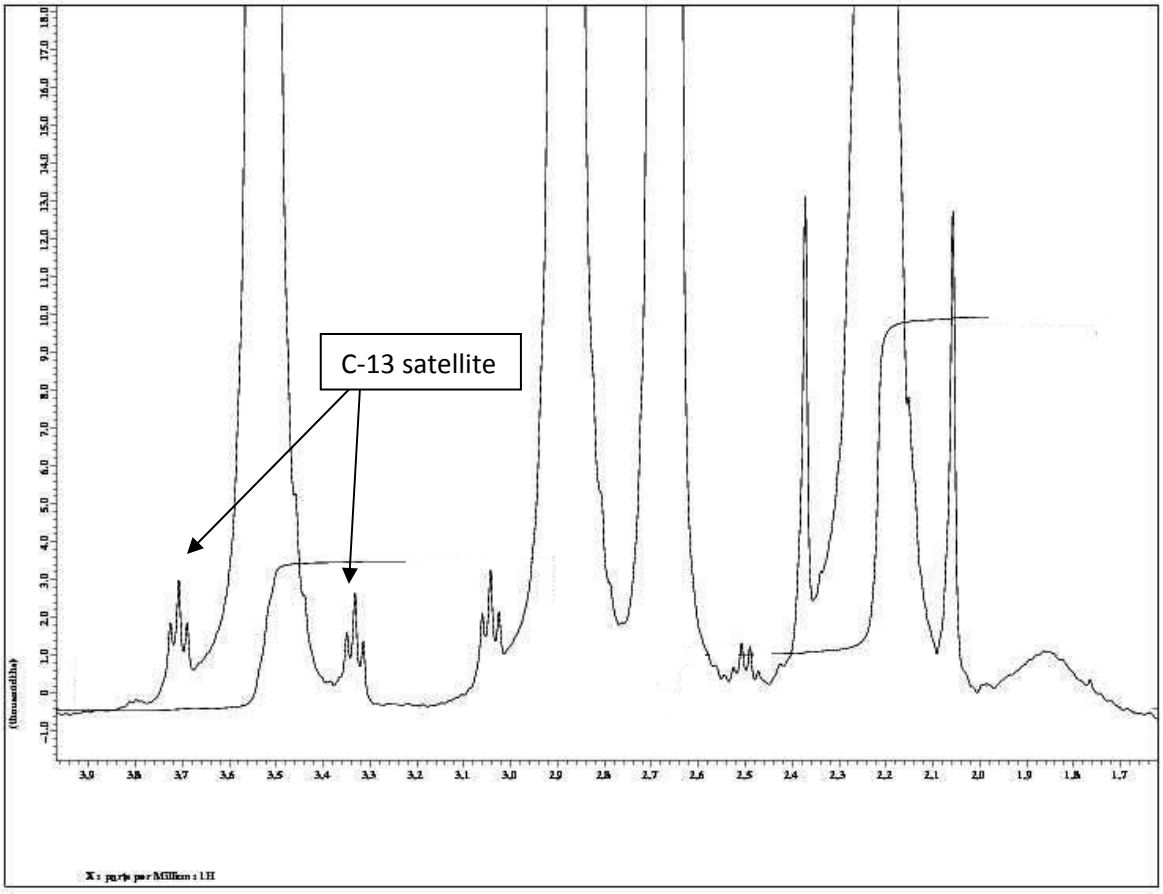

FIGURE 2: Plot showing the spectrum in Figure 1 with an expanded y-scale. The small ^{13}C satellite peaks next to the central peaks are shown. Integrals are shown. Peaks at 2.65 and 2.9 ppm are also from HN-1, but they are not baseline resolved so they were not used in the integration.

LITERATURE CITED

[1] R. J. O'Connor, M. D. Brickhouse, D. McGarvey, H. D. Durst, W. R. Creasy, J. L. Ruth, "NMR Method for the Quantitative Purity Analysis of Neat Feed Stock Samples," ECBC-TR-253, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, August 2002.

[2] M. D. Brickhouse, M. Rees, R. J. O'Connor, H. D. Durst, "Nuclear Magnetic Resonance (NMR) Analysis of Chemical Agents and Reaction Masses Produced by their Chemical Neutralization," ERDEC-TR-449, U. S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, December 1997.

[3] R. J. O'Connor, M. D. Brickhouse, J. R. Rice, H. D. Durst, D. J. McGarvey, W. R. Creasy, J. Pence, J. L. Mongomery, "NMR Method for the Quantitative Analysis of Liquid Samples," ECBC Technical Report #ECBC-TR-416, January 2005.

[4] R. J. O'Connor, M. D. Brickhouse, H. D. Durst, D. J. McGarvey, W. R. Creasy, "NMR Method for the Quantitative Analysis of VX Hydrolysates," ECBC Technical Report #ECBC-TR-417, January 2005.

[5] W. R. Creasy, D. J. McGarvey, J. S. Rice, R. J. O'Connor, and H. D. Durst, "Study of Detection Limits and Quantitation Accuracy using 300 MHz NMR," *Proceedings of the 2002 Joint Service Scientific Conference on Chemical and Biological Defense Research*, 19-21 Nov. 2002, Aberdeen Proving Ground- Edgewood Area, MD, published on CD-ROM.

[6] D. J. McGarvey and W. R. Creasy, "NIST-Traceable NMR Method to Determine Quantitative Weight Percentage Purity of Nitrogen Mustard HN-3 Feedstock Samples," ECBC-TR-1159, Aberdeen Proving Ground, MD, May 2012.

APPENDIX I: PRECISION AND ACCURACY TESTING DATA FOR THE HN-1 METHOD

I-1. APPROACH

The HN-1 purity determination method was validated using a variation of the protocol that is used in a Class I Precision and Accuracy (P&A) test. This kind of test is typically used for validation of air monitoring methods. The requirements are modified to pertain to an NMR purity determination test. A four-day test was used. On each day of the test, 10 samples and 2 blanks were prepared. The 10 samples were prepared with amounts of HN-1 of 0.2Z, 0.5Z, 0.8Z, 1.0Z, and 1.5Z, each sample in duplicate, where Z = 25 mg of HN-1. As a result, the purity method was validated for a quantity of agent of 5 mg to 37.5 mg.

The data from a P&A test is typically processed using a program called Certify (the latest version is version 6.0). Certify contains statistical criteria for the acceptance of data or the test method within acceptable measurement limits. Certify requires modifications to apply to the NMR purity determination. The target Z levels (where Z is the required detectable amount) are set in the program to be the same for all replicates from the 4-day test. For this study, the approximate target amount is measured using an adjustable pipet, and this approximate amount was chosen according to the target Z levels. For the NMR purity method, the target levels are determined by the weight of the agent that is taken from the NIST-traceable balance. So the accurate target amount is found from the weight of the agent in the vial, not from the less-accurate volume. The accurate amount cannot be entered into the Certify program as an x-coordinate.

The data that is obtained from this test easily passes the Certify pass/fail criteria of ±10%, which is much less rigorous than a purity determination requires. But because of the way the data is entered, Certify tests the accuracy of the pipeting. The actual accuracy of the data from weighing and NMR determination is much better than the Certify calculations indicate, so using only Certify to quantify the P&A results in this case would not showcase the method performance. As a result, the results are also reported in terms of standard deviations and correlation coefficients of the data.

The P&A test was performed on a JEOL ECS-400 Nuclear Magnetic Resonance Spectrometer, installed on July 2011 to meet the manufacturer's specifications. Data was collected and processed by operators who have received software and hardware training from JEOL representatives.

Weighing was done using a Sartorius Cubis balance, barcode 9804. The balance was calibrated by the ECBC Calibration Team by Reese (W959QC), identification number 27102674.

The internal standard was 1,2,4,5-tetramethylbenzene, purchased from Fluka (Sigma Aldrich), Part Number 74658-5G, CAS No. 95-93-2, as a TraceCERT® certified

reference material (CRM) standard for quantitative NMR. The lot number is #BCBC1486, Pcode number 100975857, expiration June 2012. The standard was received with a certification of analysis of 99.95% (g/g), and uncertainty of 0.08% (g/g).

The T_1 for the solutions (see Section 2.3 step e) was determined to be 2.92 s for the HN-1 peaks, and 3.18 s for the IS peak. A plot of the T_1 recovery data is shown in Figure I-1. In accordance with the measurement, 40 s was used as the NMR relaxation delay time since it is >10× the T_1 time.

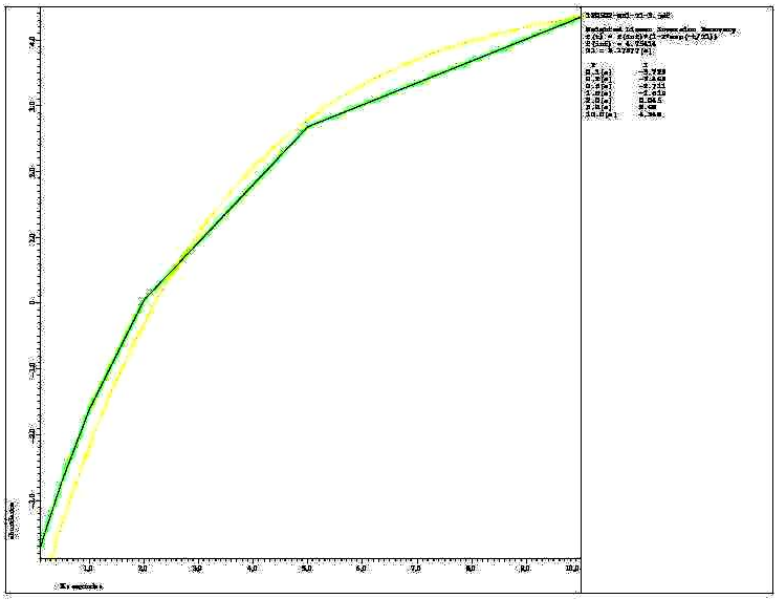

Figure I-1: Plot of NMR inversion recovery signal for the IS (green) and best exponential fit giving a T_1 value of 3.18 s (yellow).

I-2. RESULTS

Tables I-1 to I-4 show the data sets that were collected on each day of the 4-day P&A test. Figure I-2 shows the data plotted together with the regression lines and correlation coefficients.

Table I-1: P&A data from Day 1.

Z (wt agent/25 mg)	Area of Analyte	Area of Standard	Wt. Of Standard	Sample Weight	Found Z

	(agent)	(IS)			
1.4824	0.3152[a)]	0.5211[a)]	16.1500	37.0600	1.4854
1.5032	35.3745	54.4082	15.1400	37.5800	1.4968
0.9832	23.6155	44.0268	12.1000	24.5800	0.9869
1.0232	24.2895	75.2597	20.8000	25.5800	1.0208
0.8284	19.8480	58.0192	15.9400	20.7100	0.8292
0.8156	19.6222	58.9739	16.1600	20.3900	0.8176
0.5556	13.5138	54.9939	14.8400	13.8900	0.5545
0.5620	13.8943	52.2347	13.8500	14.0500	0.5602
0.2208	5.3676	59.4181	15.8500	5.5200	0.2177
0.2160	5.4671	41.3687	10.7200	5.4000	0.2154
0.0000	0.0000	56.9783	14.2700	0.0000	0.0000
0.0000	0.0000	1.0022[a)]	16.2600	0.0000	0.0000
				correlation	0.99999

a) These values were normalized differently during integration, but the ratios are correct.

Table I-2: P&A data from Day 2.

Z (wt agent/25 mg)	Area of Analyte (agent)	Area of Standard (IS)	Wt. Of Standard	Sample Weight	Found Z
1.5032	36.9333	34.7733	9.3200	37.5800	1.5052
1.5128	35.256	37.173	11.0100	37.8200	1.5879
0.9984	23.5820	35.4137	9.9100	24.9600	1.0035
0.9912	23.2531	51.6926	14.4800	24.7800	0.9905
0.7884	18.7430	39.6375	10.9700	19.7100	0.7888
0.7864	18.9870	40.527	11.1300	19.6600	0.7929
0.5056	12.2303	38.565	10.5100	12.6400	0.5068
0.4948	12.0390	38.506	10.4700	12.3700	0.4978
0.2036	4.8992	49.4986	13.3700	5.0900	0.2012
0.2052	5.0350	37.949	10.1000	5.1300	0.2038
0.0000	0.0000	39.5485	10.2000	0.0000	0.0000
0.0000	0.0000	35.605	9.4400	0.0000	0.0000
				correlation	0.99945

Table I-3: P&A data from Day 3.

Z (wt agent/25 mg)	Area of Analyte (agent)	Area of Standard (IS)	Wt. Of Standard	Sample Weight	Found Z
1.4768	36.2035	36.7571	10.3200	36.9200	1.5456
1.5040	35.1348	45.371	12.8600	37.6000	1.5143
0.9996	23.8913	35.3257	9.7200	24.9900	0.9996
0.9840	23.7482	34.3618	9.2900	24.6000	0.9763
0.8136	19.6557	34.2758	9.3000	20.3400	0.8110
0.8104	19.8095	41.7212	11.2300	20.2600	0.8108
0.5136	12.5693	49.8486	13.3700	12.8400	0.5126
0.5148	13.2879	33.3089	8.7900	12.8700	0.5332
0.1932	4.8828	29.8724	8.0100	4.8300	0.1991
0.1924	4.7809	36.0754	9.5700	4.8100	0.1929
0.0000	0.0000	61.8669	15.8500	0.0000	0.0000
0.0000	0.0000	55.5477	14.0000	0.0000	0.0000
				correlation	0.99943

Table I-4: P&A data from Day 4.

Z (wt agent/25 mg)	Area of Analyte (agent)	Area of Standard (IS)	Wt. Of Standard	Sample Weight	Found Z
1.5244	73.4158	84.8234	11.4800	38.1100	1.5109
1.4828	65.0207	121.7704	18.0200	37.0700	1.4631
1.0040	57.6180	130.7347	15.0200	25.1000	1.0066
1.0224	46.4636	82.4586	12.4100	25.5600	1.0633
0.7972	38.9671	87.5939	12.3500	19.9300	0.8354
0.8192	48.427	123.3105	14.3100	20.4800	0.8546
0.4908	23.4604	90.8508	13.0500	12.2700	0.5124
0.4968	24.3540	68.8354	9.5500	12.4200	0.5138
0.2104	10.3847	70.5217	9.8100	5.2600	0.2197
0.2000	23.2805	199.7072	11.7800	5.0000	0.2088
0.0000	0.0000	158.3831	17.8200	0.0000	0.0000
0.0000	0.0000	76.8807	9.8000	0.0000	0.0000
				correlation	0.99932

Correlation coefficients for all the days between the target Z (as a weight) and the found Z (calculated from NMR signals) are >0.999.

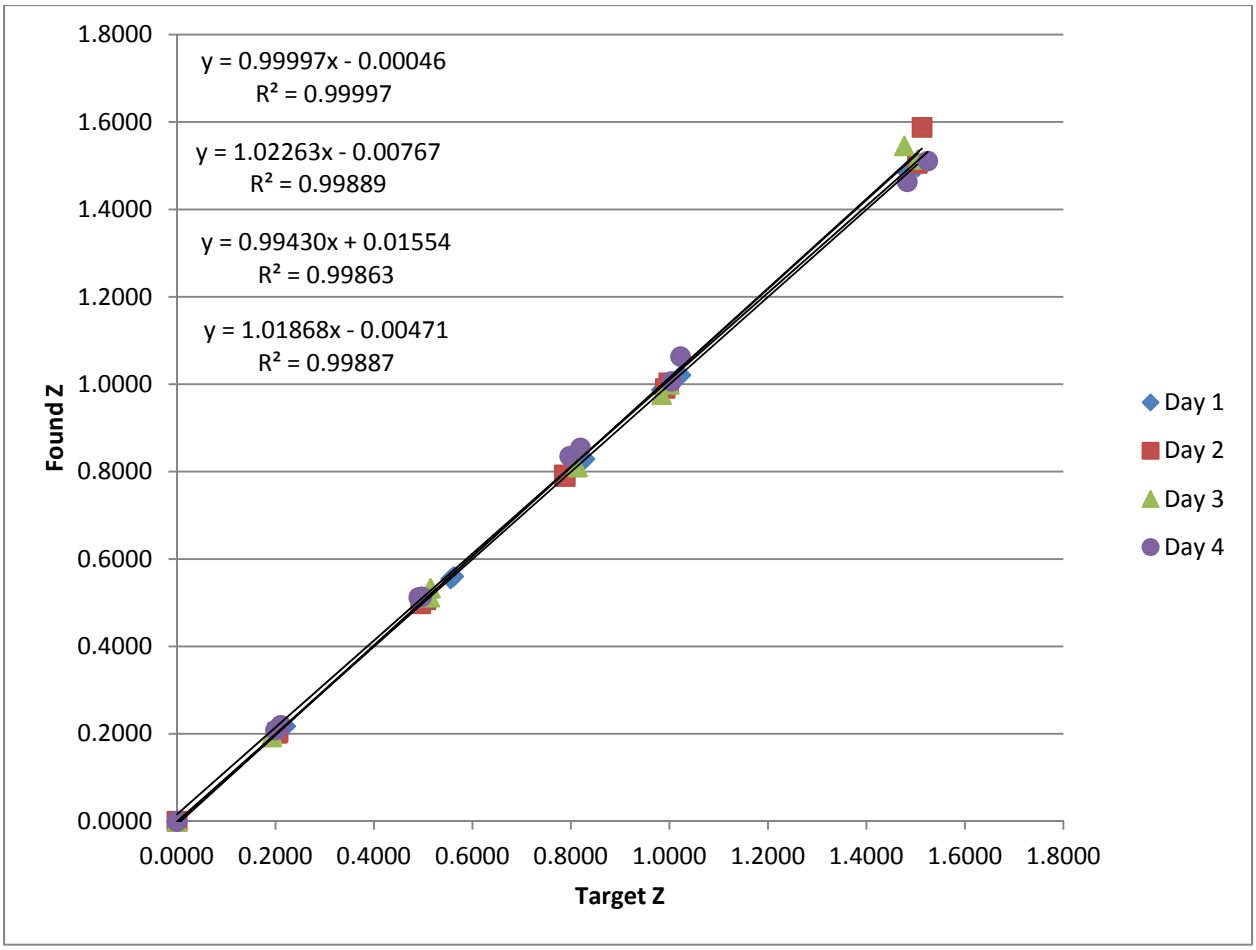

Figure I-2: Plot of the P&A data from 4 days, Found Z vs. Target Z.

In order to do the calculation for the Found Z, the formula from Section 2.5 was used, except it was normalized to 1Z = 25 mg instead of using the actual Weight A. Using this method, the purity of the HN-1 sample can be determined from the slopes of the curves from Figure I-2. Averaging all 4 slopes gives an average purity of 100.89 wt%, with a standard deviation of 1.39 wt%.

It is worth pointing out that the measurements of the weights and areas of the IS and analyte are all independent measurements, with error limits associated with them. There is no mathematical restriction from the calculations of these data that forces the purity result to be a value that is no more than 100%. (In physical terms, of course, the sample can't be more than 100% in purity.) As a result, the purity calculation can result in a number that is larger than 100%, but it should be within the statistical uncertainty of the measurements, which this result is.

The typical way to determine the purity is simply to calculate purity for each run using the formula in Section 2.5. Table I-5 shows the calculations for the Day 1 data, excluding the

blank runs. The resulting average purity is 100.01 wt%, with a standard deviation of 0.30 wt%. The 95% confidence limit is 0.59 wt%.

Table I-5: P&A data from Day 1, used to calculate purity for each run.

Area of Analyte (agent)	Area of Standard (IS)	Wt. Of Standard	Sample Weight	Weight %
0.3152	0.5211	16.1500	37.0600	100.21
35.3745	54.4082	15.1400	37.5800	99.58
23.6155	44.0268	12.1000	24.5800	100.38
24.2895	75.2597	20.8000	25.5800	99.76
19.8480	58.0192	15.9400	20.7100	100.09
19.6222	58.9739	16.1600	20.3900	100.25
13.5138	54.9939	14.8400	13.8900	99.80
13.8943	52.2347	13.8500	14.0500	99.68
5.3676	59.4181	15.8500	5.5200	98.61
5.4671	41.3687	10.7200	5.4000	99.73

Average	100.010
Standard Deviation	0.297
95% Confidence Limits	0.593

In order to minimize the amount of sample preparation, it is possible to prepare only one sample and rerun it multiple times. This approach minimizes the hazard from handling neat agent and minimizes the consumption of agent and generation of waste. However, the repetitions include only the error that is generated by the NMR data acquisition and integration, and not errors from weighing and sample preparation. Table I-6 shows data from repeated runs of the 1Z sample from Day 1.

Table I-6: Data from repeated runs of one prepared sample.

Area of Analyte (agent)	Area of Standard (IS)	Wt. Of Standard	Sample Weight	Weight %
23.3358	35.2801	9.9100	24.9600	99.83
23.6322	35.753	9.9100	24.9600	99.76
23.8347	35.6561	9.9100	24.9600	100.89
23.5779	35.6643	9.9100	24.9600	99.78
23.7549	35.598	9.9100	24.9600	100.72
23.6946	35.5785	9.9100	24.9600	100.52
23.6921	35.5534	9.9100	24.9600	100.58
23.6344	35.3756	9.9100	24.9600	100.84
Average	100.367			
Standard Deviation	0.490			
95% Confidence Limits	0.980			


EDGEWOOD CHEMICAL BIOLOGICAL CENTER
A U.S. Army RDECOM Laboratory